Formosana ruianensis n. sp. and transfer of *Oospira xinxingae* Qiu, 2022 to *Formosana* O. Boettger, 1877 (Gastropoda: Clausiliidae)

Zhi-Jie Xu[1], Ming-Wei Cai[2] & Zi-Ang He[3]

1 *Zhejiang Jinhua No. 1 Senior High School, Jinhua 321015, Zhejiang, China*
2 *Shandong Vocational Animal Science and Veterinary College, Weifang 261061, Shandong, China*
3 *Department of Senior High, Shanghai South-west Model Middle School, Xuhui 200030, Shanghai, China*
Corresponding author: *Zhi-Jie Xu (ZJXMolls@outlook.com)*

Abstract. *Formosana ruianensis* n. sp. is described from Ruian Shi, Wenzhou Shi, Zhejiang, China, and its shell and genital anatomy are illustrated. The new species was confirmed in a phylogenetic analysis based on the *16S* gene, and *p*-distances with other species of *Formosana* O. Boettger, 1877, *Formosanella* H. Nordsieck, 2003, and *Paraformosana* H. Nordsieck, 2003 are given. *Oospira xinxingae* Qiu, 2022 is transferred to *Formosana*: thus, *Formosana xinxingae* n. comb.

INTRODUCTION

Among Asian clausiliids (Gastropoda: Clausiliidae), the many species with several palatal plicae and without a tendency to form a lunella were classified in the genus *Oospira* W.T. Blanford, 1872 (Nordsieck 2001), which has resulted in the apparent high diversity of this genus. However, recent molecular studies by Motochin *et al.* (2017) and Hwang *et al.* (2022) have presented molecular evidence that disputes this character-based taxonomic treatment. Motochin *et al.* (2017) revealed that both nuclear and mitochondrial DNA show that *Oospira* and some of its subgenera, including *Formosanella* H. Nordsieck, 2003 and *Formosana* O. Boettger, 1877, are unrelated. Additional evidence that Taiwanese *Formosana* are distinct from *Oospira* was given by Hwang *et al.* (2022) in their thorough study of Taiwanese *Formosana* based on molecular, morphological, and anatomical data. As a result, Nordsieck (2021) adopted this separation of *Formosana* from *Oospira*.

In recent decades, several new Chinese species of clausiliids have been described. So far, fieldwork and studies of Asian clausiliids have discovered numerous new species (Nordsieck 2001, 2003, 2005, 2007, 2016; Grego & Szekeres 2011, 2017; Hunyadi & Szekeres 2016), but within the limits of relatively few collections and collectors, as well as the lack of anatomical data. Many species are endemic to small areas. Some regions of China, such as Zhejiang Province, have not had sufficient fieldwork and may still have unreported new species, as Nordsieck (2012) had revealed the rich diversity of Chinese clausiliids.

One of us (Ming-Wei Cai) collected specimens of an unknown species of *Formosana* in Wenzhou City, Zhejiang Province, China, which we later identified as a new species. This taxon is most like *Formosanella friniana* (Heude, 1890) and the Taiwanese *Formosana* species. We describe and figure the characters of the shell and genitalia, and we undertook a phylogenetic analysis using the *16S* gene. We classify our new species in the genus *Formosana* on account of its morphology and the results of this phylogenetic analysis. Additionally, we transfer *Oospira xinxingae* Qiu, 2022 to the genus *Formosana*, based on characters of its shell and genitalia, as well as its distribution.

MATERIALS AND METHODS

Thirteen specimens of the new species were collected, all from the type locality. Two living specimens were found. Geographic coordinates were obtained using Bigemap GIS software (http://www.bigemap.com/) with Map World as the satellite image source (https://www.tianditu.gov.cn/). In the locality data, *zhen* means "town" and *shi* and *xian* means "city".

One specimen was dissected for anatomical study, while the other was used for in molecular analysis. We also sequenced samples of another five species; the polymerase chain reaction (PCR) primers and methods used are shown in Table 1. The PCR primers were synthesized by Sangon Biotech (Shanghai, China). We used PhyloSuite v. 1.2.3 (Zhang *et al.* 2020; Xiang *et al.* 2023) and MEGA v. 11 (Tamura *et al.* 2021) for our phylogenetic analysis. Other sequences from Hwang *et al.* (2022) and Mamos *et al.* (2021) were downloaded from GenBank (Table 2), and *Grandinenia ardouiniana* (Heude, 1885) was used as the outgroup. Sequences were aligned using Muscle in MEGA v. 11 and checked and clipped manually. PhyloSuite v. 1.2.3 (Zhang *et al.* 2020) was used to conduct, manage, and streamline the analyses with the help of several plug-ins.

Gap sites were removed with trimAl v. 1.2 rev. 57 (Capella-Gutiérrez *et al.* 2009) using the "automated1" command. The clipped *16S* sequences were ultimately 255 base pairs long. To generate a Bayesian tree, the best partitioning scheme and evolutionary models for 1 predefined partition were selected using PartitionFinder2 v. 2.1.1 (Lanfear *et al.* 2016) with the greedy algorithm and small-sample-corrected Akaike Information Criterion (AICc). Bayesian-in-ference phylogenies were inferred using MrBayes v. 3.2.7a (Ronquist *et al.* 2012) under the partition model (2 parallel runs, 5 million generations), in which the initial 25% of sampled data were discarded as burn-in. To generate a maximum-likelihood tree, ModelFinder v. 2.2.0 (Kalyaanamoorthy *et al.* 2017) was used to select the best-fit model using the Bayesian information criterion (BIC). Maximum-likelihood phylogenies were inferred using IQ-TREE v. 2.2.0 (Nguyen *et al.* 2015) under the K81u+G4+F model for 20,000 ultrafast bootstraps (Minh *et al.* 2013), as well as the Shimodaira–Hasegawa-like approximate likelihood-ratio test (Guindon *et al.* 2010). The results were visualized in Fig-Tree v. 1.4.4 (https://tree.bio.ed.ac.uk/software/figtree/) and Adobe Illustrator 2022. We calculated *p*-distances using the clipped sequence in MEGA v. 11, with 1,000 bootstrap replications and a 50% coverage cutoff.

The holotype specimen of the new species was deposited in the mollusc collection of the Museum of Hebei University (HBUMM, Baoding, Hebei). Paratypes are in the private collections of Zhi-Jie Xu (ZJXC, Jinhua, China), Shi-Ji Peng (SJPC, Chengdu, China), Zheng-Ping Liu (ZPLC, Chengdu, China), Li-Wen Lin (LWLC, Xiamen, China), Zi-An Qiu (ZAQC, Nanjing, China), Zhong-Guang Chen (ZGCC, Nanchang, China), Jin-Cheng Lu (JCLC, Shang-

Table 1. Primer pairs and PCR conditions used in the analyses of the *16S* rRNA gene.

Primer pairs (5′ to 3′)	16SAR CGCCTGTTTATCAAAAACAT
	16SBR CCGGTCTGAACTCAGATCACGT
Reaction systems	25 µl Robust PCR MasterMix ×2, 1 µl each primer, 2 µl DNA, 16 µl double-distilled H$_2$O
Cycling conditions	94 °C for 30 s, 94 °C for 10 s, 45 °C for 50 s, 72 °C for 110 s, 40 cycles; 72 °C for 10 min.
Reference	Palumbi *et al.* 1991

Table 2. GenBank accession numbers of sequences used in this paper.

Species	Locality	GenBank accession no.	Source
Formosana ruianensis n. sp.	Ruian Shi, Wenzhou Shi, Zhejiang Province, China	OR600921	This study
Formosanella bensoni (H. Adams, 1870)	Fengdu Xian, Chongqing Shi, China	OR600922	This study
Formosana cf. *pacifica* (Gredler, 1884)	Qingyuan Shi, Guangdong Province, China	OR778285	This study
Formosana pacifica (Gredler, 1884)	Chagsha Shi, Hunan Province, China	OR778286	This study
Formosana cf. *malleolata* H. Nordsieck, 2012	Lingchuan Xian, Guilin Shi, Guangxi Province, China	OR778284	This study
Paraformosana indurata (Heude, 1887)	Wulong District, Chongqing Shi, China	OR770171	This study
Formosana formosensis (H. Adams, 1866)	Butterfly Valley, Kaohsiung Shi, Taiwan	MW378971	Hwang *et al.* 2022
Formosana swinhoei (L. Pfeiffer, 1866)	Dawulun, Keelung Shi, Taiwan	MW378933	Hwang *et al.* 2022
Formosana taiwanica (Pilsbry, 1909)	Lija, Taidong Shi, Taiwan	OL470453	Hwang *et al.* 2022
Formosana lingchuanensis K.-M. Chang, 1989	Simaxian, Miaoli Xian, Tawan	OL470428	Hwang *et al.* 2022
Formosana albiapex K.-M. Chang & Ookubo, 1994	Mount Lilongshan, Pingdong Shi, Taiwan	OL470402	Hwang *et al.* 2022
Formosanella friniana (Heude, 1890)	Lingyin Temple, Hangzhou Shi, Zhejiang, China	OL470454	Hwang et al. 2022
Grandinenia ardouiniana (Heude, 1885)	Mount Poem, Xialong City, Vietnam	MW378932	Mamos *et al.* 2021

hai, China), and Forest City Studio (FCSC, Shaghai, China).

We follow Nordsieck's (2012) concept of the genera *Formosanella* and *Formosana*.

Results

Bayesian- and maximum-likelihood trees (Fig. 1) show similar topology. Among all the nodes in the Bayesian tree, two nodes are not supported by a posterior probability of >0.5. The posterior probability at each node range from 0.3 to 0.97. Posterior probabilities <0.9 appear in eight nodes, while the remaining two nodes are >0.9. In the maximum-likelihood tree, the bootstrap values of the nodes range from 43 to 90. Only the value of one node is <50, and one is >90.

All Taiwanese *Formosana* species form a terminal cluster with high support (maximum-likelihood bootstrap support, BS = 90; Bayesian posterior probability, BPP = 0.94). The nodes of other Chinese species of *Formosana*, *Formosanella*, and *Paraformosana* have low support (BS ≤ 73, BPP ≤ 0.86), and *Formosanella* species are at the root of the tree. All *Formosana* species form a cluster with a low-support value (BS = 63, BPP = 0.58). These findings are consistent with the results of Hwang *et al.* (2022). The *p*-distances among species analyzed is shown in Table 3.

Systematics

Family Clausiliidae Gray, 1855

Subfamily Phaedusinae A.J. Wagner, 1922

Genus *Formosana* O. Boettger, 1877

Type species. *Clausilia swinhoei* L. Pfeiffer, 1866, by original designation.

Remarks. Genital characters (Fig. 2) differ between this genus and *Formosanella* H. Nordsieck, 2003. All *Formosana* species with known genitalia characters have a fat, strong penis and epiphallus, while in the genitalia of *Formosanella bensoni* and *Formosanella recedens* these are rather thinner.

Formosana ruianensis Xu & Cai, n. sp.
Figures 1, 2C, 3–5

ZooBank identifier. urn:lsid:zoobank.org:act:005C0173-3A13-43C1-94A3-68FB04397F70

Diagnosis. A large (26.0–28.7 mm high), fat *Formosana* species with a large, conical protoconch and subcolumellar lamella receding behind peristome. Palatal plicae 7–9. Aperture detached from penultimate whorl.
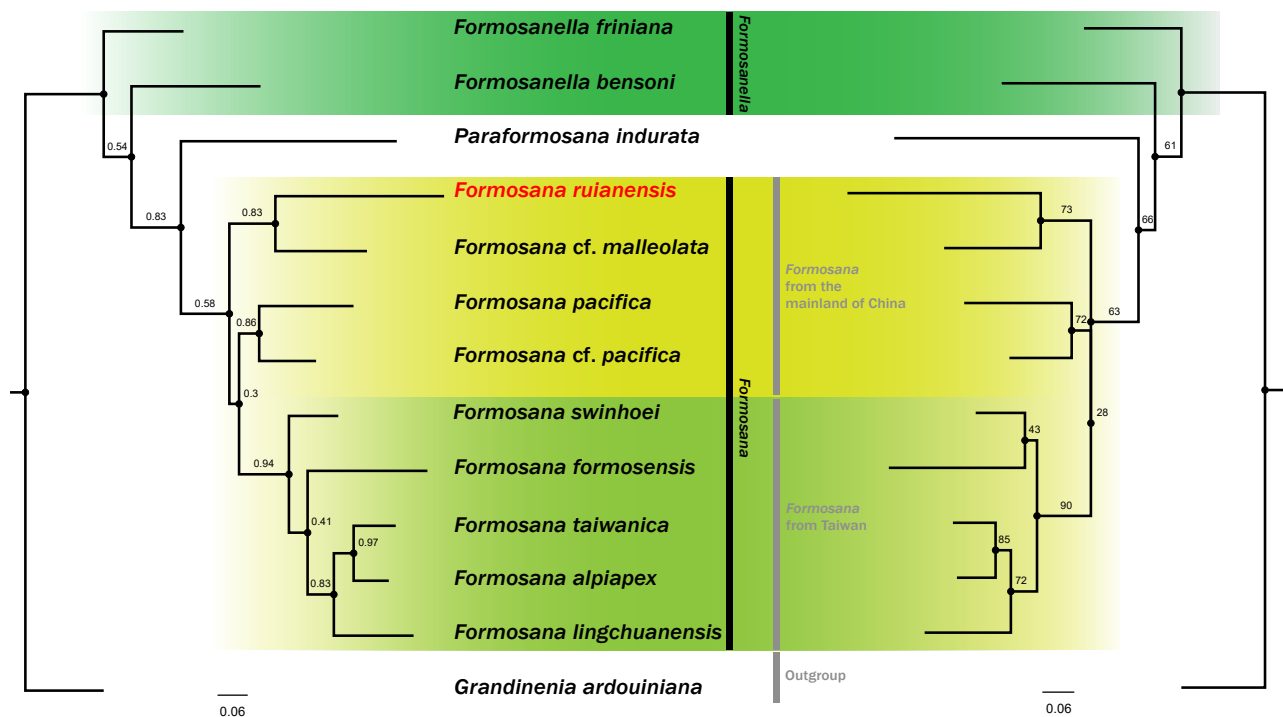

Figure 1. Bayesian-inference tree (left) and maximum-likelihood tree (right) inferred from *16S* gene sequences. Bayesian posterior probabilities and bootstrap supports are shown on the left/right nodes. The new species is shown in red.

Table 3. Genetic distance (*p*-distances) between *Formosana*, *Formosanella*, and *Paraformosana* species of the *16S* gene (lower left of the matrix) and standard error (upper right of the matrix).

		1	2	3	4	5	6	7	8	9	10	11	12
1	*Formosana ruianensis* n. sp.		0.027	0.028	0.029	0.028	0.027	0.028	0.028	0.028	0.028	0.028	0.029
2	*Formosana* cf. *malleolata*	0.246		0.026	0.027	0.028	0.027	0.027	0.027	0.028	0.028	0.029	0.028
3	*Formosana* cf. *pacifica*	0.265	0.215		0.025	0.027	0.027	0.027	0.025	0.027	0.026	0.027	0.028
4	*Formosana pacifica*	0.303	0.236	0.189		0.028	0.027	0.027	0.026	0.027	0.027	0.029	0.028
5	*Formosana formosensis*	0.273	0.264	0.219	0.257		0.027	0.020	0.023	0.024	0.028	0.028	0.029
6	*Formosana swinhoei*	0.267	0.250	0.233	0.251	0.241		0.025	0.026	0.026	0.028	0.029	0.028
7	*Formosana taiwanica*	0.270	0.233	0.231	0.246	0.116	0.202		0.023	0.023	0.028	0.029	0.028
8	*Formosana lingchuanensis*	0.269	0.236	0.202	0.225	0.149	0.209	0.152		0.025	0.028	0.028	0.028
9	*Formosana albiapex*	0.290	0.257	0.236	0.238	0.180	0.213	0.166	0.204		0.028	0.028	0.029
10	*Formosanella friniana*	0.287	0.269	0.213	0.255	0.269	0.263	0.294	0.265	0.266		0.026	0.028
11	*Formosanella bensoni*	0.273	0.291	0.249	0.291	0.253	0.291	0.310	0.264	0.274	0.219		0.028
12	*Paraformosana indurata*	0.309	0.262	0.266	0.268	0.279	0.272	0.275	0.266	0.287	0.272	0.273	
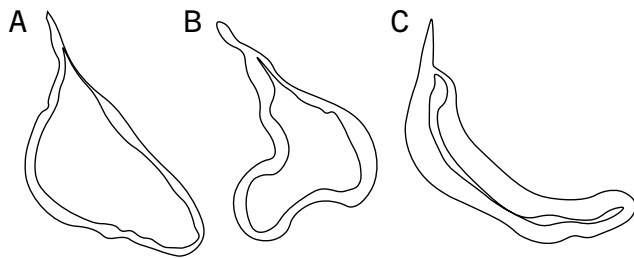
Figure 2. Penis and epiphallus of *Formosanella bensoni* (**A**), *Formosanella recedens* (**B**), and *Formosana ruianensis* (**C**). Not to scale. (Illustrations by Zhi-Jie Xu.)

Type locality. Mountains near Gaolou Zhen [高楼镇], Ruian Shi [瑞安市], Wenzhou Shi [温州市], Zhejiang Province, China (27.735° N, 120.287° E).

Type materials. Holotype: leg. Ming-Wei Cai, 6 April 2023, HBUMM 08774 (shell and separate, ethanol-preserved body). Paratypes: 11 dead-collected shells, from same locality and date as holotype (ZJXC/4, SJPC/1, ZPLC/1, LWLC/1, ZAQC/1, ZGCC/1, JCLC/1, FCSC/1); 1 live-collected specimen (ZJXC/1), from same locality, leg. Ming-Wei Cai, 25 October 2023 (shell and separated ethanol-preserved body).

Distribution. Known only from the type locality.

Description. Shell (Fig. 3A–C), fat, spindle-shaped, yellowish-brown. Whorls 8–10. Protoconch relatively large, conical, not attenuated, without decollation, and with fine dense striae, both spirally and axial, which gives shell a silk texture. Neck rounded, with striae slightly stronger and wavy. Aperture pyriform. Peristome thick, detached, and expanded, its edge pale brownish, reflexed. Superior lamella

(Fig. 3E) high; junction between its inner end and spiral lamella marked by an angle. Inferior lamella strong, reaching peristome margin, steeply ascending. Subcolumellar lamella weakly emerging, only visible in oblique view. Palatal plicae 6–8, dorsolateral in position, with uppermost one longest (Fig. 3D). Clausilium plate not visible in oblique view.

Shell measurements. Holotype: shell height 26.0 mm, shell width 7.5 mm, aperture height 7.5 mm, aperture width 6.0 mm. Paratypes: shell height 27.3–28.7 mm (mean 27.19 mm), shell width 7.3–8.1 mm (mean 7.64 mm), aperture height 6.1–7.5 mm (mean 7.02 mm), aperture width 5.0–6.4 mm (mean 5.9 mm).

Genitalia (Fig. 4). Penis thickened, its appearance simple, smooth, its length ½ that of spermoviduct and hermaphroditic duct combined. Epiphallus fusiform, thickened, shorter than penis. Vas deferens short, <1/7 of length of epiphallus. Penial-retractor muscle inserted at approximately ⅓ length of epiphallus at end with vas deferens. Bursa copulatrix ellipsoid. Diverticulum adherent to spermoviduct.

Etymology. The species is named for Ruian Shi, which is the city near the type locality.

Chinese vernacular name. 瑞安丽管螺 (Pinyin: ruì ān lì guǎn luó).

Habitat. Living snails (Fig. 5A, B) were found in a deciduous forest in an area of igneous rock, under damp leaf litter or on damp moss.

Remarks. The holotype animal was observed to be oviparous when temporarily being raised by one of us (Ming-Wei Cai).

Comparison. The overall appearance and protoconch whorls

Figure 3. *Formosana ruianensis* n. sp. **A**, **B**, holotype (HBUMM 08774). **C**, paratype in Li-Wen Lin collection. **D**, **E**, details of plicae and lamellae, respectively (not to scale). (Photographs by Li-Wen Lin; illustration by Zhi-Jie Xu.)
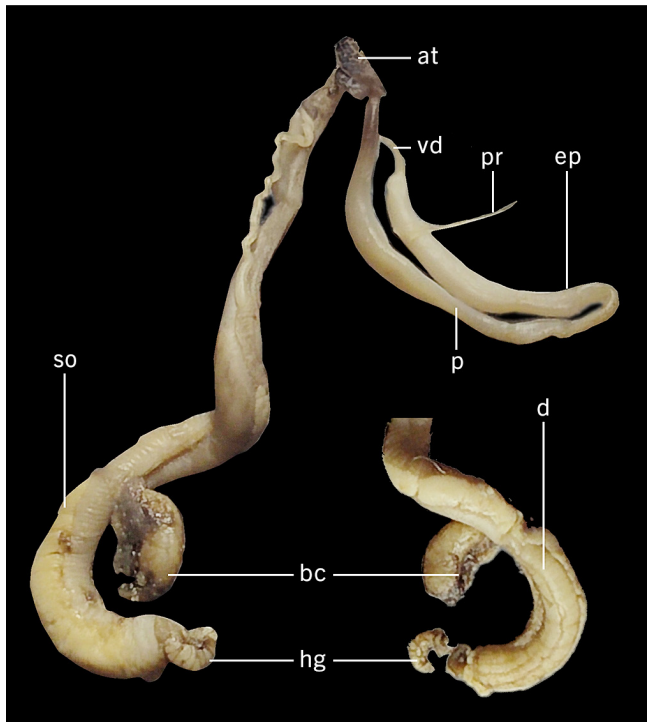
Figure 4. Genitalia of *Formosana ruianensis* n. sp. (not to scale). Abbreviations: at = atrium, bc = bursa copulatrix, d = diverticulum, ep = epiphallus, hg = hermaphroditic gland, p = penis; pr = penis-retractor muscle, so = spermoviduct; vd = vas deferens. (Photographs by Zhi-Jie Xu.)

of this species are similar to Taiwanese species of *Formosana*. In both *F. formosensis* (H. Adams, 1866) and *F. swinhoei* (L. Pfeiffer, 1866), the protoconch is attenuate, the peristome is adpressed to the last whorl, and the subcolumellar lamella reaches to the peristome. In the new species, the protoconch is not attenuated, the peristome is detached from the last whorl, and the subcolumellar lamella is not visible from a frontal view. Our phylogenetic analysis indicates that *Formosana ruianensis* n. sp. is distinct, with a basal position in the clade *Formosana*, from species of mainland China, while Taiwanese *Formosana* species form a terminal cluster. From Hwang *et al.*'s (2022) anatomical study, we conclude that all Taiwanese species of *Formosana* in which the anatomy is known have a relatively long vas deferens, while *Formosana* from mainland China for which genitalia are known—*F. jianyueae* (Chen, 2020), *F. xinxingae* (Qiu, 2022) n. comb., and *F. ruianensis* Xu & Cai, n. sp.—have the vas deferens distinctly shorter according to dissections by Chen (2020), Qiu (2022), and us.

We compared the new species with *F. pacifica* (Gredler, 1884) and two other similar species, *F. malleolata* H. Nordsieck, 2012 and *F. splendens* H. Nordsieck, 2005. All these species have adpressed peristomes and attenuate protoconchs. *Formosana pacifica* is distinctly smaller than the new species (Yen 1939; Nordsieck 2005) with a similar shell

Figure 5. Living specimens and habitats of *Formosana ruianensis* n. sp. **A**, paratype, coll. Zhi-Jie Xu. **B**, holotype (HBUMM 08774). **C**, overview of the habitat. (Photographs by Ming-Wei Cai.)

height (25.2 mm) but much smaller shell width (4.9 mm) (measured by us), while in the other two species the subcolumellar lamella reaches the peristome and is visible in frontal view. Some specimens of *F. malleolata* and *F. splendens* have a doubled peristome, while the type specimens of *F. ruianensis*, we found no doubled peristome.

Nordsieck (2003) originally placed *Formosanella* as a subgenus of *Oospira* and diagnosed it as having a steeply ascending inferior lamella. Hwang *et al.* (2022) included *Formosanella friniana* in this genus based on only phylogenetic evidence, showing that morphological characters of the shell cannot clearly differentiate *Formosanella* and *Formosana*. Therefore, although the new species is biogeographically closest to *Formosana*, and shares some shell characters (palatal plicae and the shape of inferior lamellae) with it, we classify the new species as *Formosana* based on our phylogenetic analysis of the *16S* gene.

Formosana xinxingae (Qiu, 2022) n. comb.

Oospira xinxingae Qiu 2022: 3, figs 1–3.

Remarks. This species was originally described as a species of *Oospira* in the sense of Nordsieck (2007). However, now that *Oospira* has been divided, the generic placement of this species should be reconsidered. In Qiu's (2022) original description, this species was said to be most like *Oospira magnanciana* (Heude, 1882), now *Formosana magnanciana* (Heude, 1882), in characters of both the shell and genitalia. We also note that it shares a simple penis and epiphallus, as well as a short vas deferens, with *F. jianyueae* and *F. ruianensis* Xu & Cai, n. sp. *Formosana xinxingae* occurs in Chongqing, rather than Yunnan, Guangdong, and Guizhou, where *Oospira* species are found (Nordsieck 2012). Thus, we consider that "*Oospira*" *xinxingae* belongs in *Formosana*.

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